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Genetics

Genes encoding α and β subunits of Na,K-ATPase are located on three different chromosomes in the mouse

(α-subunit isoforms/gene mapping)

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ABSTRACT **We have made use of a panel of mouse–hamster somatic cell hybrids and restriction fragment length polymorphisms between two mouse species (*Mus musculus* and *Mus spretus*) to determine the chromosomal localization of genes encoding the α and β subunits of the Na,K-ATPase (Na^+,K^+-activated ATP phosphohydrolase, EC 3.6.1.3). DNA probes for three distinct isoforms of the Na,K-ATPase α subunit mapped to three different mouse chromosomes: the α_1 gene (*Atpa-1*) cosegregated with the *Egf* gene on chromosome 3; α_2 (*Atpa-2*) with the cytochrome P-450PB gene family/coumarin hydroxylase locus on chromosome 7; α_3 (*Atpa-3*) with the α-spectrin gene on chromosome 1. The Na,K-ATPase β-subunit gene (*Atpb*) mapped to the same region of chromosome 1, but it was not tightly linked to the *Atpa-3* gene. These results indicate that three isoforms of the Na,K-ATPase α subunit are encoded by three distinct genes. The dispersion of Na,K-ATPase genes suggests that their expression is not likely to be controlled by a common cis-acting regulatory element.**

The Na,K-ATPase (Na^+,K^+-activated ATP phosphohydrolase, EC 3.6.1.3) is a membrane-bound enzyme responsible for the active transport of the Na^+ and K^+ in most animal cells. The enzyme has been shown to consist of two subunits. The α subunit is a polypeptide of M_r 100,000, which contains the catalytic site for ATP hydrolysis (1). The β subunit is a glycosylated polypeptide of M_r 55,000, whose function has not yet been established. Multiple forms of the Na,K-ATPase have been described in a variety of animal species (2, 3). Two biochemically distinct isoforms of the catalytic subunit (α and α^+) have been identified in rat brain (2). These differ in electrophoretic mobility in $NaDodSO_4$-containing gels (2, 4) and in sensitivity to cardiac glycosides (2, 5). However, the molecular basis for α-subunit diversity has not been clearly explained.

We and others have recently isolated cDNA clones for three distinct isoforms of the rat Na,K-ATPase α subunit (6, 7). Comparison of the deduced amino acid sequence of the α-subunit isoforms revealed that the polypeptides are highly conserved and share >85% amino acid sequence homology (6, 7). Significant differences in codon usage and in the pattern of genomic DNA hybridization indicated that multiple α-subunit isoforms could be encoded by different genes (6). Rat Na,K-ATPase β-subunit cDNA clones have also been characterized (8, 25), and multiple forms of β-subunit mRNA have been identified. These mRNAs appear to be derived from a single gene by alternative RNA splicing (8, 25).

To further investigate the molecular basis for Na,K-ATPase isoform diversity, we have determined the chromosomal positions of the mouse genes encoding the α and β subunits of the enzyme by two complementary approaches. First, the segregation of genomic DNA sequences in mouse–hamster somatic cell hybrids has been used to identify a chromosomal assignment for each gene. Second, the segregation of restriction fragment length polymorphisms (RFLPs) in N_1 backcross progeny of a *Mus musculus* × *Mus spretus* mating has been used to confirm chromosome assignment. Using this approach, we have established linkage between each ATPase subunit gene and another marker previously assigned to the same chromosome (9). The gene encoding the α_1 subunit (*Atpa-1*) of the ATPase, which is expressed in all tissues tested thus far, has been assigned to mouse chromosome 3. The α_2 subunit of the ATPase, also designated αIII (7), is expressed predominantly in brain and fetal heart (6). The gene encoding this subunit (*Atpa-2*) has been assigned to mouse chromosome 7. The α_3 or α^+ (7) subunit gene (*Atpa-3*) of the ATPase encodes two distinct mRNA species 4.5 and 6 kilobases (kb) long. The smaller mRNA species is present in a variety of tissue types, while the 6-kb species is found only in brain, heart, and skeletal muscle (6). The gene encoding the α_3 subunit is located on mouse chromosome 1. The Na,K-ATPase β-subunit gene (*Atpb*), which also exhibits a distinctive and complex tissue-specific pattern of expression (8, 25), maps to the same region of chromosome 1 but is not tightly linked to the α_3-subunit gene. The dispersion of the three α-subunit genes demonstrates that this subunit of the Na,K-ATPase is encoded by a multigene family.‖

MATERIALS AND METHODS

Somatic Cell Hybrids. Mouse–Chinese hamster somatic cell hybrids (EBS) were derived from the fusion of BALB/c mouse spleen cells with Chinese hamster V79 lung fibroblasts (clone E36) (11).

Chromosome Composition of Cell Hybrids. Mouse chromosomes were identified in cell hybrids by karyotyping and examining hybrid cells in the same cell passage for mouse chromosome-specific enzyme markers (11). To confirm the analyses for mouse chromosomes 1–7, genomic DNA from the hybrid cells was filter hybridized as described below using markers previously assigned to the respective chromosomes.

Mouse Strains. The F_1 females from several *M. musculus* × *M. spretus* matings and their N_1 backcross progeny were obtained from Oak Ridge National Laboratories. BALB/c

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Abbreviation: RFLP, restriction fragment length polymorphism.

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‖Mouse genes encoding the Na,K-ATPase subunits have been named according to the rules for mouse gene nomenclature as stated (10).

and C57BL/6 animals were obtained from The Jackson Laboratory.

Analysis of Genomic DNA. High molecular weight DNA was prepared from mouse–hamster somatic hybrid cells and from mouse tissues by the method of Milbrandt *et al.* (12). Hybrid cell, BALB/c, and E36 DNA samples were digested with *Eco*RI or *Hin*dIII, while N_1 backcross and accompanying control DNA samples were digested with *Taq* I or *Hin*dIII. Restriction enzyme digestion products were separated by electrophoresis on 1% agarose gels containing 0.04 M Tris acetate (pH 8) and 0.002 M disodium EDTA. Separated DNA fragments were transferred to reusable hybridization membranes (Zetabind, AMF Cuno). Capillary transfer was done for 16 hr in the presence of 1.5 M NaCl/0.15 M sodium citrate. The blots were hybridized for 36 hr in the presence of 50% formamide/0.75 M NaCl/75 mM sodium citrate/1× concentrated Denhardt's solution (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone)/25 mM sodium phosphate, pH 6.8/500 μg of denatured salmon sperm DNA per ml with 10^7 cpm (<50 ng) of the indicated hybridization probe. The following Na,K-ATPase subunit probes were used: clone rb5 [1.2-kilobase-pair (kbp) rat cDNA encoding portion of the α_1 subunit] (13), clone rb2 (2.0-kbp *Eco*RI/*Stu* I fragment of the rat cDNA encoding the amino-terminal portion of the α_2 subunit) (6), clone rb13c (Rα3-a, 2.7-kbp rat cDNA encoding portion of the α_3 subunit) (6), and clone rb19 (900-bp rat cDNA encoding portion of the β subunit) (8). Radiolabeled DNA probes were synthesized with the Klenow fragment of DNA polymerase I with random hexanucleotides (P-L Biochemicals) and [α-^{32}P]dCTP (14). Blots were washed to a final stringency of 30 mM NaCl/3 mM sodium citrate/0.1% $NaDodSO_4$ at 55°C and exposed to Kodak XAR-5 films for 24 hr to 2 wk at −80°C with an intensifying screen.

RESULTS

Assignment of the α_1-Subunit Gene (*Atpa-1*) to Chromosome 3. The α_1-subunit cDNA was hybridized to DNA derived from a panel of somatic cell hybrids in which segregation of mouse chromosomes has occurred on a hamster background. This probe hybridizes with high intensity to three genomic DNA fragments (5.7, 3.7, and 2.5 kbp long) in *Eco*RI-digested mouse DNA (Fig. 1*A*, BALB/c lane). Hybridization to DNA fragments of these three sizes is observed in a number of the hybrid cell lines (EBS1, EBS5, EBS9, EBS13, EBS74, EBS2A, EBS2Ag, EBS 15A, EBS 18A, and EBS 51A). In all other cell lines tested, hybridization is not observed at any of the three positions. These results indicate that these three *Eco*RI fragments are located on the same chromosome. Inspection of the data presented in Table 1 indicates that the gene encoding the α_1 subunit segregated concordantly with chromosome 3 and discordantly with all other chromosomes. These data therefore suggest assignment of the *Atpa-1* gene to mouse chromosome 3.

To further investigate the position of the *Atpa-1* gene in the mouse genome, the segregation of a RFLP between *M. musculus* and *M. spretus* for the *Atpa-1* gene was followed in a series of N_1 backcross animals. Cosegregation was observed between the RFLP for *Egf*, a locus previously assigned to mouse chromosome 3 (15), and the *Atpa-1* gene RFLP in 23 of 32 backcross animals (Table 2). These results provide further support for the assignment of the *Atpa-1* gene to chromosome 3 and suggest that it is located ≈28 map units from the gene encoding epidermal growth factor. It is of note that the difference in ouabain sensitivity between rodent and primate cells has been previously assigned to mouse chromosome 3 in somatic cell genetic experiments (16). We suggest that this concordance reflects the encoding of a ouabain-resistant ATPase α subunit by the *Atpa-1* locus on mouse chromosome 3.

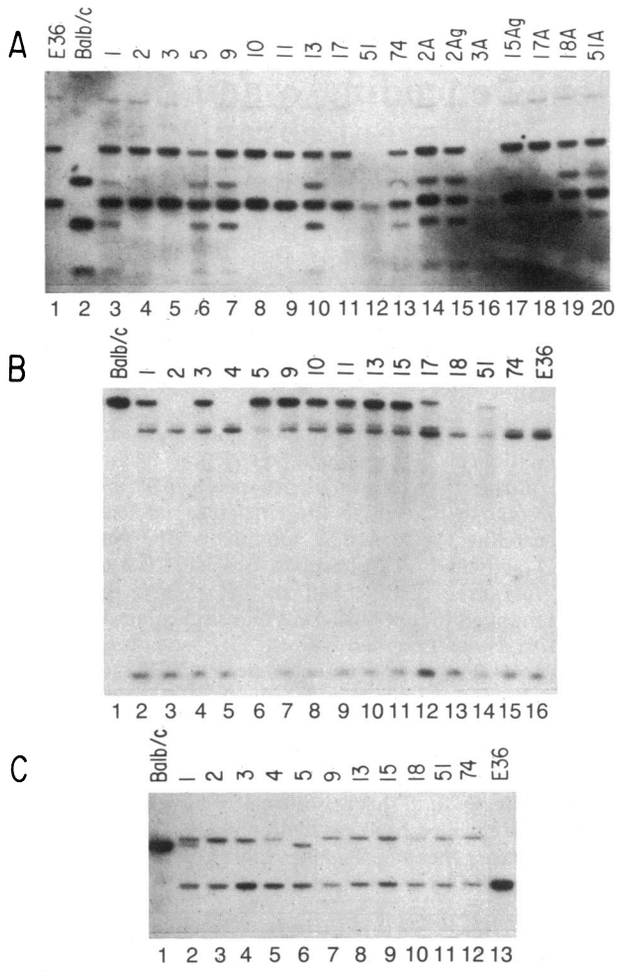

FIG. 1. Na,K-ATPase α_1- (*A*), α_2- (*B*), and β- (*C*) subunit genes in Chinese hamster and mouse cells and cell hybrids. DNA digested with *Eco*RI. (*A*) Hamster DNA contains two major fragments (8.4 and 4.4 kbp) that hybridize to the rb5 probe; mouse DNA contains 5.7-, 3.7-, and 2.5-kbp hybridizing fragments. Lanes: 1, hamster control; 2, mouse control; 3–20, EBS hybrids. EBS lines 1, 5, 9, 13, 74, 2A, 2Ag, 15Ag, 18A, and 51A are positive; all other lines are negative. (*B*) Hamster DNA contains three fragments (14.5, 13, and 2 kbp) that hybridize to the rb2 probe; mouse DNA contains a 22-kbp hybridizing fragment. Lanes: 1, mouse control; 2–15, EBS hybrids; 16, hamster control. EBS lines 1, 3, 5, 9, 10, 11, 13, 15, and 51 are positive; all others are negative. In EBS17 (lane 12) the fragment >22 kbp is a product of partial *Eco*RI restriction of hamster α_2-subunit gene DNA. (*C*) Hamster DNA contains a 4.7-kbp fragment that hybridizes to the rb19 probe. However, the hybrid lines contain two hybridizing fragments (7.3 and 4.7 kbp), except for EBS5. Mouse DNA contains a 6.6-kbp hybridizing fragment. Lanes: 1, mouse control; 2–12, EBS hybrids; 13, hamster control. EBS lines 1 and 5 are positive; all others negative.

Assignment of the α_2-Subunit Gene (*Atpa-2*). To localize the gene encoding the α_2 subunit of the ATPase, a probe specific for the α_2-subunit cDNA was hybridized to the somatic cell hybrid panel (Fig. 1*B*). An *Eco*RI fragment 22 kbp long was the only DNA sequence hybridizing to the probe in *Eco*RI-digested BALB/c mouse DNA. Hybridization at this position was observed in 9 of the 14 hybrid cell lines tested. Examination of the discordancies between the presence and absence of each mouse chromosome and the presence or absence of hybridization (Table 1) led to the conclusion that the *Atpa-2* subunit gene was likely to be located on mouse chromosome 7.

Table 1. Discordancies in chromosome localization of Na,K-ATPase subunit genes using mouse–hamster hybrid cell lines

Subunit gene	Cell lines tested	Mouse chromosome																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
Atpa-1	18	7	6	0	5	6	3	6	3	8	5	10	5	8	6	8	4	4	6	3	10
Atpa-2	14	7	4	5	4	6	7	0	4	8	3	9	4	5	9	4	7	4	5	3	5
Atpa-3	17	0	7	6	5	7	6	7	3	6	6	7	7	6	3	10	6	4	7	3	12
Atpb	11	0	7	4	3	3	4	5	3	4	2	2	5	6	2	8	4	5	4	4	9

Cell hybrids retaining different mouse chromosomes have been described (11). Presence or absence of mouse chromosomes 1–7 was verified by probing hybrid cell DNA with the following probes: chromosome 1, α-spectrin; chromosome 2, catalase and follicle-stimulating hormone; chromosome 3, epidermal growth factor and nerve growth factor; chromosome 4, v-*mos* and pronatriodilatin/atrial natriuretic factor; chromosome 5, α-fetoprotein and serum albumin; chromosome 6, T-cell receptor β chain; chromosome 7, cytochrome P-450b. Genes were determined by scoring the presence or absence of the respective gene fragments on DNA blots. Concordant cell lines have either retained or lost a Na,K-ATPase-specific subunit gene together with a specific mouse chromosome. Discordant cell lines have either retained a subunit gene but not a specific chromosome or vice versa. Chromosome assignment is based on concordant segregation and the absence of discordancies.

To test this assignment, the segregation of a RFLP identified by the α_2-subunit cDNA was compared to the segregation of other markers assigned to chromosome 7. Cosegregation was observed in 32 of 32 N_1 backcross animals between the *Atpa-2* locus and a RFLP identified by the cytochrome P-450b cDNA (17) (Table 2). The P-450b cDNA recognizes the major phenobarbital-inducible cytochrome P-450 (*P-450PB*) gene family, which maps to the coumarin hydroxylase (*Coh*) locus on mouse chromosome 7 (18, 19). This result confirms the assignment of the α_2-subunit gene to mouse chromosome 7 and localizes this gene to the same region of the chromosome as the cytochrome P-450PB/coumarin hydroxylase locus.

Assignment of the α_3-Subunit Gene (*Atpa-3*). To identify the chromosomal location of the α_3-subunit gene, the segregation of a mouse-specific restriction fragment was followed in the mouse–hamster hybrid panel. Results of these hybridization studies are summarized in Table 1. No discordancies were observed between the presence or absence of chromosome 1 and the presence or absence of the mouse-specific restriction fragment hybridizing to the α_3-subunit cDNA. Since at least three discordancies were observed for all other mouse chromosomes, the *Atpa-3* gene was tentatively assigned to chromosome 1.

To further test this assignment, the segregation of a RFLP between *M. spretus* and *M. musculus* identified by the α_3-subunit cDNA probe (Fig. 2) was compared to the segregation of a number of other chromosome 1 markers, including α-spectrin (20, 21). As indicated in Table 2, close linkage between the structural locus encoding α-spectrin (*Spta-1*) and the *Atpa-3* gene was established. Thirty-one of 31 N_1 animals tested showed cosegregation of these two markers. These results establish that *Atpa-3* is located in close proximity to the α-spectrin gene on chromosome 1.

Table 2. Linkage of Na,K-ATPase subunit genes to known markers on mouse chromosomes determined by RFLP analysis of DNA from *M. musculus* × *M. spretus* N_1 backcross animals

Subunit gene	Mouse chromosome	Markers tested	Cosegregants/animals	% recombinants
Atpa-1	3	*Egf*	23/32	28
Atpa-2	7	*P-450b/Coh*	32/32	0
Atpa-3	1	*Spta-1*	31/31	0
Atpb	1	*Spta-1*	29/31	7

For *Atpa-1*, *Atpa-2*, *Atpa-3*, *Atpb*, *Egf*, and *P-450b*, *Taq* I RFLPs between *M. musculus* and *M. spretus* were analyzed. For α-spectrin (*Spta-1*), a *Hin*dIII RFLP was analyzed.

Assignment of the β-Subunit Gene (*Atpb*). To identify the chromosomal location of the β-subunit gene, the segregation of a mouse-specific restriction fragment identified by the cDNA for the β subunit in mouse–hamster hybrids was determined. As shown in Fig. 1*C*, the mouse-specific *Eco*RI fragment of 6.6 kbp is present in cell lines EBS1 and EBS5. This pattern of segregation is identical to that observed for the *Atpa-3* gene. As observed for *Atpa-3*, only chromosome 1 showed no discordancies when compared to the segregation pattern for the β-subunit gene.

To determine whether the β-subunit gene was closely associated with the *Atpa-3* gene and to further test the assignment to chromosome 1, segregation of a β-subunit gene RFLP in the *M. spretus* × *M. musculus* backcross was tested. As indicated in Table 2, 29 of 31 N_1 animals showed

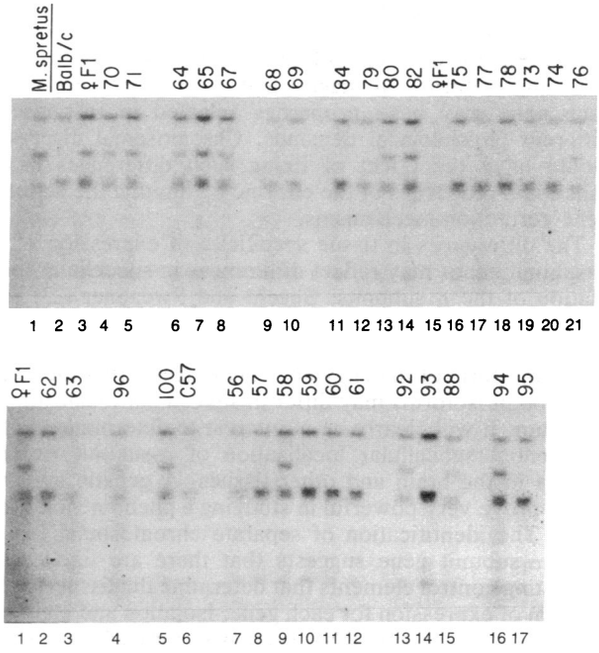

FIG. 2. Na,K-ATPase α_3-subunit DNA in *M. spretus*, *M. musculus*, F_1 cross, and N_1 backcross animal cells. DNA was digested with *Taq* I. Lane 1, *M. spretus* control; lane 2 (*Upper*) and lane 6 (*Lower*), *M. musculus* controls; lanes 3 and 15 (*Upper*) and lane 1 (*Lower*), F_1 females; lanes 4–14 and 16–21 (*Upper*) and lanes 2–5 and 7–17 (*Lower*), N_1 animals. N_1 animals having both *M. musculus* and *M. spretus* alleles are lines 70, 71, 64, 65, 67, 80, 82, 76, 96, 100, 58, 92, 88, and 94. All others are homozygous for the *M. musculus* allele.

cosegregation of the β-subunit gene and the α-spectrin gene. This result confirms the assignment of *Atpb* to mouse chromosome 1. However, the result also indicates that *Atpa-3* and *Atpb* are located at a substantial distance from each other on the chromosome. The likelihood that a cis-acting control element is involved in the coordination of expression of these two genes is therefore not high.

DISCUSSION

The complementary use of somatic cell genetics and an N_1 interspecies backcross has provided a very efficient strategy for determining the map position of each gene encoding a Na,K-ATPase subunit. Each strategy has been used independently in previous studies for gene mapping in the mouse (9, 15). The conjunction of the two approaches provides additional confidence regarding both chromosomal assignment and the position of the gene on the chromosome.

The distribution of genes encoding alternative forms of a polypeptide on different chromosomes has been observed for a number of multigene families. Genes encoding alternative forms of actin and myosin show a chromosomal distribution of this type (9). The structurally related subunits of the acetylcholine receptor are also encoded by chromosomally dispersed genes (22). The localization of genes encoding three isoforms of the α subunit of the Na,K-ATPase to different mouse chromosomes raises interesting questions regarding the evolutionary history of this multigene family. The deduced amino acid sequence for each isoform of the α subunit indicates a very high degree of evolutionary conservation. Complete identity is observed at 825 of 1013 positions in the amino acid sequence for all three isoforms. This degree of homology strongly indicates a common ancestral origin for these three genes. The fact that each gene is located on a different chromosome makes gene correction mechanisms that can operate on a tandem array of duplicated genes unlikely for the α-subunit gene family. The selective pressures that have operated on each gene independently must therefore have been quite high. However, it is also important to note that the differences in tissue distribution of the three α-subunit isoforms (6) suggest that the enzyme encoded by each gene may have properties selected in response to different physiological demands. Chromosomal dispersion would have the effect of fixing such differences in the functional properties of the enzyme by limiting the action of gene correction mechanisms.

The differences in tissue specificity of expression of the α-subunit genes may reflect differences in subcellular localization of the α subunits. Specht and Sweadner (23) have presented evidence that the α^+-subunit form of the enzyme exhibits increased levels of axonal transport compared to the α-subunit form of the enzyme. This suggests that the α- and α^+-subunit isoforms may differ in subcellular localization in rat brain. It will clearly be of interest to determine whether differential subcellular localization of α-subunit isoforms occurs in the brain and other tissues. A genetic approach could prove very powerful in studying a phenomenon of this type. The identification of separate chromosomal loci for each α-subunit gene suggests that there are likely to be cis-acting control elements that determine the tissue-specific pattern of expression for each gene. Isolation and characterization of the genomic DNA sequences for each gene should permit the identification of the control elements that encode this specificity.

The localization of the *Atpb* gene raises important questions regarding the overall control of Na,K-ATPase expression. A number of lines of evidence suggest that α and β subunits are expressed at approximately equal levels (8, 24). It is important to consider that the β-subunit gene is located at a distance from any presently mapped α-subunit gene. This would suggest that coordinate regulation of the subunits must be carried out through trans-acting mechanisms. A further issue that should be raised in this context is the observation that the β subunit exhibits a tissue-specific pattern of expression distinct from any of the α-subunit genes (6, 8, 13). If relatively equal levels of expression of α- and β-subunit genes are required for ATPase activity, then it may be possible that the β-subunit gene may also be a member of a multigene family. In the liver in particular, the level of expression of the only β-subunit gene that has been identified to date is substantially lower than the level of expression of the α_1-subunit gene (8, 13). If a liver-specific β-subunit gene is identified, it will be of interest to determine whether it maps in close proximity to the β-subunit gene mapped in this study or at a different chromosomal locus.

The localization of the genes for each subunit brings up an additional question of interest. Is it possible that defects in the function of one or more of these genes produce a recognizable phenotype in the mouse? The mapping of these genes to specific chromosomal positions opens the prospect of determining whether previously described or newly identified mouse mutant phenotypes may be due to defects in ATPase function.

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